Indian Journal of Agricultural Sciences **86** (2): 208—15, February 2016/Article
https://doi.org/10.56093/ijas.v86i2.55960

Diversity of culturable gut bacteria associated with the field populations of cotton leafhopper (*Amrasca biguttula biguttula*) in India

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Received: 26 November 2014; Accepted: 12 August 2015

ABSTRACT

Field populations of cotton leafhopper [*Amrasca biguttula biguttula* (Ishida)] exposed to heavy applications of imidacloprid, acephate, acetamiprid and dimethoate were collected from the seven cotton (*Gossypium* spp) growing areas of the country. Thirty culturable bacteria were isolated from the guts of 16 populations of leafhoppers and were characterized through morphological and molecular methods. A good diversity of microflora was recorded across the location and is unique with respect to the locations. None of them are repeated except the genera *Bacillus* and *Enterobacter.* There was more number of gut microflora associated with the leafhoppers collected from Dharwad where the insecticide usage pattern and the number of sprays were very high as compared to other locations. Various *Bacillus* spp. were reported in the Dharwad population. The association of *Enterococcus asburiae, Enterobacter silesiacus* from the guts of leafhoppers of Guntur which was exposed to nine rounds of sprays of acephate, imidacloprid and dimethoate as compared to Bangalore which was not exposed to insecticides at all. *Enterococcus hormaechei* was isolated from the insects of Bangalore. The predominant bacterial genera identified in *A.biguttula biguttula* were *Serratia*, *Bacillus, Enterococcus, Enterobacter, Pantoea, Methylobacterium, Stenotrophomonas, Pseudomonas* and *Paenibacillus.*

Key words: 16S rDNA sequences, *Amrasca biguttula biguttula* microflora, Diversity, Phylogenetic analysis

Cotton (*Gossypium* spp.) is an important fibre crop grown world over in 111 countries. In India cotton is cultivated in 11.7 M ha with a production of 39.09 M bales of 170 kg (Anonymous 2015). Peshin *et al.* (2009) reported that *Amrasca biguttula biguttula* (Ishida) (Hemiptera: Cicadellidae) is an alarming pest of cotton, causing yield loss greater than 100-114 kg of lint /ha.The nymphs and adults suck the sap from leaves and cause phytotoxic symptoms (hopper burn) which results in complete desiccation of plants. Farmers use high pesticide dose, i.e. 6 to 7 rounds for a single crop of 150-180 days duration (Banerjee *et al.* 2000). The introduction of Bt-cotton in India in 2002, enabled reduction of insecticide sprays for bollworms, however this indirectly caused resurgence of sucking pests specially leafhoppers (Kranthi 2007). The cotton leafhopper developed resistance to the recommended neonicotinoids, organophosphates (Praveen *et al*. 2006, Ram Singh and Jaglan 2005, Kshirsagar *et al.* 2012). Indiscriminate use of insecticides can result in insect resistance to insecticides.

Microbes present in the alimentary canals of insects play a role in insecticide resistance (Werren 2012). The gut microflora also play a significant role in the host insect morphogenesis, food digestion, nutrition, antifungal toxin production, pheromone production, regulation of pH, synthesis of vitamins, temperature tolerance, resistance against parasitoid development, and detoxification of noxious compounds (Genta *et al*. 2006). A wide array of gut microflora especially yeasts and bacteria were isolated and characterized from *Aphis* spp., *Chrysoperla zastrowi sillemi, Cotesia plutellae, Trichogramma* spp and *Helicoverpa armigera* (Srinivasamurthy *et al*. 2011, Madhusudan *et al*. 2011, Hemalatha *et al*. 2012, Sneha *et al*. 2013) and they studied the role of these microflora in fitness attributes such as insecticide resistance, fecundity etc. In insect pest management programs, the development of resistance to insecticides mediated through detoxifying enzymes like glutathione-S-transferase is a major constraint (Sarfraz *et al.* 2006, Mohan and Gujar 2003). These detoxifying enzymes have been acquired by insects through bacteria during the course of evolution (Vuilleumier 1997). The role of gut bacterial enzymes of host insect in insecticidal resistance development has been documented (Boush and Matsumura 1967, Indiragandhi *et al*. 2007 and

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Table 1 Identification of gut bacteria associated with *A. biguttula biguttula*

Geographical locations	Population(s) collected (Insecticide usage pattern)*	Number of rounds of spray	Strain code	GenBank accession	Identified organism	Identity match (%)	Farmer's perception on the efficacy of the insecticide (% of leaf-hopper controlled)
Guntur 16°18' N 80°26' E	3 (Ap + Dm; Ap+ Imi; Ap + Imi)	9	CLHG- 1	KC425474	*Staphylococcus pasteuri*	100	13
			CLHG-1a	KC427093	*Enterococcus silesiacus*	99	
			CLHG- 2	KC428704	*Bacillus amyloliquefaciens*	100	
			YCLHG-2	KC603555	*Enterobacter asburiae*	97	
Ludhiana 30°54' N 75°51' E	2 (Ap + Am; Ap + Imi)	4	CLHPAUL-1	JX893010	*Serratia marcescens*	99	20
			CLHPAUP-3	JX893012	*Lysinibacillus sphaericus*	99	
			CLHPAUP-4	JX893013	*Proteus mirabilis*	100	
Dharmapuri 18°56' N 79°05' E	2 (Am; Imi)	8	CLHDB 1-1	KC603557	*Ralstonia pickettii*	97	12
			CLHDB 2-2	KC603560	*Bacillus anthracis*	99	
			CLHDF 1-2	KC603566	*Methylobacterium komagatae*	97	
			CLHDF 2-3	KC603558	*Ralstonia solanacearum*	98	
			CLHDF 2-1	KC858856	*Agrobacterium* sp.	99	
Dharwad 15°27' N 75°00' E	5(Ap+Imi; Imi+Dm; Imi+Am; Imi+Imi; Am)	10	CLHDHA (1)F 2-1	KC603561	*Bacillus megaterium*	99	10
			CLHDHA (2)F-2	KC603559	*Erwinia persicina*	99	
			CLHDG(1) B 1-1	KC603570	*Pseudomonas geniculata*	97	
			CLHDG(2) B 1-1	KC603569	*Stenotrophomonas maltophilia*		98
			CLHDU(2) B 1-1	KC603571	*Massilia varians*	98	
			CLHDU(1) F 1-1	KC603567	*Bacillus atrophaeus*	99	
			CLHDU (2) B 1-2	KC858858	*Hymenobacter gelipurpurascens*	98	
			CLHDHF (2) B 2-2	KC858849	*Staphylococcus gallinarum*	99	
			CLHDHF (2)B 1-1	KC858851	*Bacillus cereus*	99	
			CLHDHF (2)B 2-1	KC858859	*Bacillus subtilis*	100	
			CLHDHF (2)F 2-2	KC858860	*Brevibacterium halotolerans*	98	
Bangalore 13°06' N 77°33' E	1 (No insecticide)		LHBAF-1	KC858852	*Enterobacter hormaechei*	99	0
			LHBAB-2	KC858853	*Microbacterium oxydans*	99	
Raichur 16°12' N 77°21' E	1 (Ap+ Imi)	8	CLHRB-2	KC858862	*Xanthomonas* sp.	99	15
Almora 25°24' N 81°54' E	1 (Dm)	5	CLHH 2-2	KC465363	*Phenylobacterium* sp.	99	25
			YBLHG-2	KC603556	*Paenibacillus cineris*	98	
Someshwar 25°24' N 81°51' E	1 (Ap)	6	CLHS-1	KC465360	*Exiguobacterium* sp.	100	18
			CLHSYPD-1	KC603554	*Klebsiella variicola*	99	

Robyn *et al*. 2011). As evidenced by the above works the role played by microflora in insecticide resistance and host fitness, this study was conceived to characterize the diversity of culturable microflora associated with the cotton leafhopper *A.biguttula biguttula* to assess their role in insecticide resistance. Identifying the interactions between the insects and their gut bacteria with respect to insecticide resistance may provide the way for novel approaches for insect control. No attempts have been made so far across the world to study the gut bacteria associated with the insecticide exposed insect *A. biguttula biguttula.* Hence the present study was undertaken to characterize and document the gut bacteria associated with the insecticide exposed leafhopper *A. biguttula biguttula*, which may be explored for their role in insecticide resistance and other host fitness attributes.

MATERIALS AND METHODS

Sixteen populations of adults of *A. biguttula biguttula* were collected with the help of an aspirator and collection tubes from insecticide (imidacloprid, acephate, acetamiprid and dimethoate) sprayed fields of cotton from various locations of the country, viz. Dharmapuri, Dharwad, Guntur, Ludhiana, Almora, Raichur and Someswar of India (Table 2). Information pertaining to number of sprays, insecticide use pattern against the leafhopper and their efficacy on the control of insects were also collected from the farmers. The collected insects were categorised based on insecticide use pattern of different locations. Gut microflora were isolated as per the standard procedure described by Feng *et al*. (2011). The appendages of leafhoppers, viz. head, wings and legs were carefully removed with sterile blades and the remaining body was surface sterilized with 0.1% NaOCl for 60 s followed by 70% ethanol twice for 1 min and then rinsed thoroughly with sterile distilled water. The body was macerated with the help of sterile mini pestle and mortar. The gut contents were put in 10 ml sterile water blank and the contents were swirled and dilutions were prepared up to 10^{-3}. Aliquots of 100 µL of the diluted content was spread onto four growth media, viz. Potato Dextrose Agar (PDA), Nutrient Agar (NA), Luria Bertani Agar (LB) and Yeast Peptone Dextrose Agar (YPDA). The plates were incubated for 48 hr at 30 °C and observed every 24 hr for the development of microbial colonies. The individual bacterial colonies were purified through spread plate technique and maintained in 50% glycerol. The purified

Table 2 Morphological characters of gut bacteria associated with *A. biguttula biguttula*

Microorganisms	Form	Elevation	Margin	Colour	Gram's reaction	Shape
Staphylococcus pasteuri	Circular	Raised	Entire	Yellow	+	Cocci
Enterococcus silesiacus	Circular	Raised	Entire	White	+	Cocci
Bacillus amyloliquefaciens	Irregular	Flat	Undulate	White	+	Rod
Enterobacter asburiae	Circular	Raised	Entire	White	-	Rod
Serratia marcescens	Circular	Raised	Entire	Red	-	Rod
Lysinibacillus sphaericus	Circular	Raised	Entire	White	+	Rod
Proteus mirabilis	Irregular	Raised	Undulate	White	-	Rod
Ralstonia pickettii	Circular	Raised	Entire	Buff	-	Rod
Bacillus anthracis	Irregular	Flat	Undulate	White	+	Rod
Methylobacterium komagatae	Circular	Raised	Entire	Pink	-	Rod
Ralstonia solanacearum	Circular	Raised	Entire	Buff	-	Rod
Agrobacterium sp.	Circular	Convex	Entire	Yellow	-	Rod
Bacillus megaterium	Irregular	Flat	Undulate	White	+	Rod
Erwinia persicina	Irregular	Raised	Undulate	Cream	-	Rod
Pseudomonas geniculata	Circular	Raised	Entire	Yellow	+	Rod
Stenotrophomonas maltophilia	Circular	Flat	Entire	White	-	Rod
Naxibacter varians	Circular	Flat	Entire	White	-	Rod
Bacillus atrophaeus	Irregular	Flat	Undulate	White	+	Rod
Hymenobacter gelipurpurascens	Circular	Convex	Entire	White	-	Rod
Staphylococcus gallinarum	Circular	Convex	Entire	Yellow	+	Cocci
Bacillus cereus	Irregular	Flat	Undulate	White	+	Rod
Bacillus subtilis	Irregular	Flat	Undulate	White	+	Rod
Brevibacterium halotolerans	Circular	Convex	Entire	White	+	Rod
Enterobacter hormaechei	Circular	Convex	Entire	White	-	Rod
Microbacterium oxydans	Circular	Convex	Entire	White	+	Rod
Xanthomonas sp.	Circular	Flat	Entire	Cream	-	Rod
Phenylobacterium sp	Circular	Convex	Entire	Yellow	-	Rod
Paenibacillus cineris	Irregular	Convex	Undulate	Buff	-	Rod
Exiguobacterium sp.	Circular	Elevated	Entire	Orange	+	Rod
Klebsiella variicola	Circular	Convex	Entire	White	-	Rod

bacterial cultures were revived in a nutrient broth and were characterized using morphological and molecular methods.

The pure cultures of all the bacteria were grown on nutrient agar medium and characterized based on standard morphological characters described by Harley and Prescott (2002) and Gram's reaction.

DNA extraction of all the isolated bacterial strains was carried out with the help of HiPurA^{TM} Bacterial and Yeast Genomic DNA Purification Spin Kit procured from HiMedia Pvt. Ltd. The isolated genomic DNA was amplified using the forward primer pA-5'AGAGTTTGATCCTGGCTCAG3' and reverse primer pH-5'AAGGAGGTGATCCAGCCGCA3'. The amplifications were carried with the following reaction mixture: 8µl DNA template, 4µl dNTP's (10Mm), 2µl of each primer, 0.8 µl Taq DNA Polymerase, 5µl Taq buffer B, 3µl $MgCl_2$, 25.2 µl of molecular grade water. Amplification reactions were carried out in a Quantarus PCR system apparatus under the following conditions : an initial denaturation of 3 min at 94ºC , followed by 35 cycles of denaturation at 94ºC for 1 min , annealing for 1 min at 45ºC and an extension for 2 min at 72ºC. An extra extension step of 10 min at 72ºC was added after completion of the 35 cycles. Amplification of 16S rRNA gene by PCR resulted in a product of 1.5 kb in size for all microbial strains. PCR products were sequenced directly with the *Taq*- mediated dideoxy chain terminator cycle sequencing in ABI 3130*xl* automated genetic analyser (Applied Biosystem, UK) as per manufacturer's instructions. The contiguous sequences were formed from forward and reverse sequences using online CAP3 programme. The contiguous sequences were used for homology search of the 16S rDNA sequences using the Blast N with the sequences deposited in (Genbank, NCBI). The identification were based on percentage similarity (>97% compared with NCBI database), by BLAST homology.

Phylogenetic analysis was performed by taking into consideration the 16s rDNA sequences of the bacterial strains isolated and the sequences available in public databases (Genbank, NCBI) that have maximum identity with the isolated strains. A phylogenetic tree was constructed using molecular evolutionary genetic analysis using MEGA version 5.2 of Tamura *et al*. (2011), after multiple alignment by CLUSTAL W (Thompson *et al*. 1994). Closely related sequences were used in constructing the tree using maximum likelihood algorithm and Kimura-2 parameter corrections described (Kimura 1980). The statistical confidence of the nodes was estimated by using bootstrap replication of 850 described by Felsenstein (1985). Sequences obtained were submitted to NCBI database and accession numbers were obtained (Table 1).

RESULTS AND DISCUSSION

Characterization of microflora

The morphological characters of the colony *viz*., form, elevation, margin and colour were recorded for all isolated microflora (Table 2). The colonies of majority of the bacteria were circular, entire and raised; fourteen were observed as Gram positive and sixteen were Gram negative (Table 2).

Thirty culturable gut bacteria associated with sixteen field populations of cotton leafhopper *A. biguttula biguttula* were isolated (Table 2) and identified through 16S rDNA sequences with the available bacterial sequences (closest representatives) in public database (GenBank, NCBI). The nucleotide sequences of the collected bacterial strains were subjected to homology searches in DNA databases, which revealed that the sequences of *Bacillus amyloliquefaciens*, *B. subtilis*, *Exiguobacterium* sp., *Proteus mirabilis*, *Staphylococcus pasteuri* of field caught population showed 100% similarity with the 16S rRNA gene sequences of the respective identified organism, while *Agrobacterium* sp., *Bacillus anthracis*, *B. atrophaeus*, *B. cereus*, *B. megaterium*, *Enterobacter hormaechei*, *Enterococcus silesiacus*, *Erwinia persicina*, *Klebsiella variicola*, *Lysinibacillus sphaericus*, *Microbacterium oxydans*, *Phenylobacterium* sp., *Serratia marcescens*, *Staphylococcus gallinarum*, *Xanthomonas* sp. showed 99% similarity. *Brevibacterium halotolerans*, *Hymenobacter gelipurpurascens*, *Massilia varians*, *Paenibacillus cineris*, *Ralstonia solanacearum*, *Stenotrophomonas maltophilia* showed 98% similarity and *Enterobacter asburiae*, *Methylobacterium komagatae*, *Pseudomonas geniculata*, *Ralstonia pickettii* showed 97% similarity.

Genotypic diversity and phylogenetic analysis

Phylogenetic analysis was carried out for their similarity to known bacteria aligned together with the sequences (closest representatives), available in public databases (GenBank, NCBI), of bacteria (Fig 1). Three genetic groups were formed among 30 representative bacterial strains. Group I included 18 isolates (*Agrobacterium* sp., *Bacillus amyloliquefaciens*, *B.anthracis*, *B.megaterium*, *B.atrophaeus*, *B.cereus*, *B.subtilis*, *Brevibacterium halotolerans*, *Enterococcus silesiacus*, *Exiguobacterium* sp., *Hymenobacter gelipurpurascens*, *Lysinibacillus sphaericus*, *Methylobacterium komagatae*, *Microbacterium oxydans*, *Paenibacillus cineris*, *Phenylobacterium* sp., *Staphylococcus pasteuri* and *S. gallinarum*). Group II included 8 isolates (*Enterobacter asburiae*, *E. hormaechei*, *Erwinia persicina*, *Klebsiella variicola*, *Massilia varians*, *Proteus mirabilis*, *Ralstonia solanacearum* and *Serratia marcescens*). Group III included 3 isolates *(Stenotrophomonas maltophilia, Pseudomonas geniculata* and *Xanthomonas* sp.). Two isolates were out-grouped from other isolates namely *Ralstonia pickettii* and *Stenotrophomonas maltophilia* (Fig 1). It was observed that there were more number of gut microflora associated with the insects of Dharwad (11 nos) where the insecticide usage pattern and the number of sprays were very high as compared to other locations, viz. Guntur (4 nos), Ludhiana (3 nos), Dharmapuri (5 nos), Raichur (1 no), Almora (2 nos) and Someshwar (2 nos) (Table 1). It was interesting to observe that the perception of the farmers with respect to the efficacy of insecticides on control of leafhoppers ranged

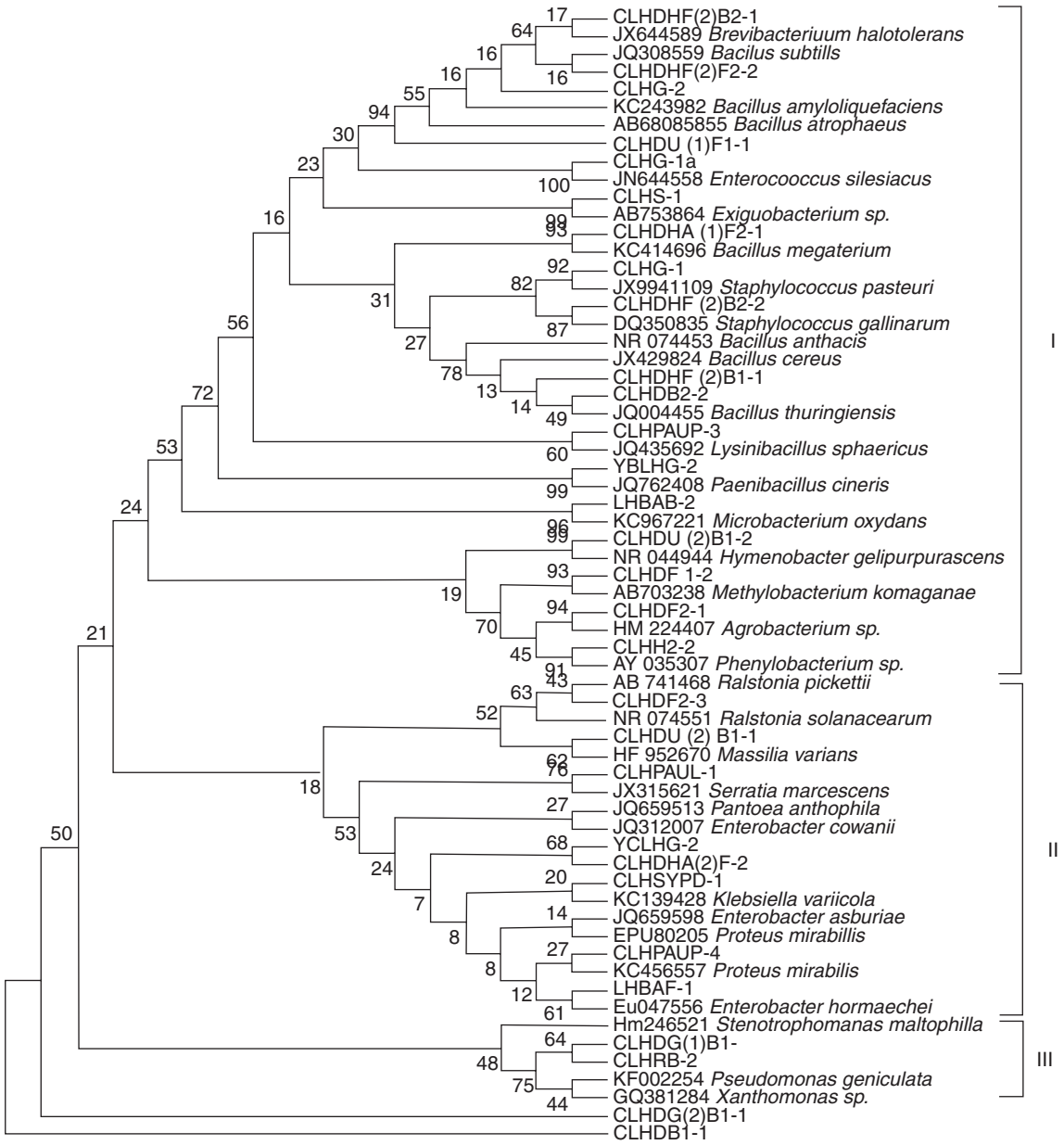

Fig 1 Molecular Phylogenetic analysis by Maximum Likelihood method using 16S rRNA gene sequences identified from sequences deposited in GenBank. The numbers at branch points of the tree designate boot strap values

from 10 to 25 % (Table 2).

We observed the incidence of leafhopper in the farmer's fields even after the repeated sprays of various combination of insecticides and they felt that insecticides miserably failed in controlling the leafhoppers and the farmers' perception was very low. The field observations followed by interaction with farmers indicated that the insects might have developed resistance against the insecticides. Development of resistance is a common problem due to use of a single insecticide or insecticides with a common mode of action, against populations for consecutive generations. Peshin *et al*. (2009) stated that globally about 504 insects are known to have developed resistance against insecticides and is a dynamic phenomenon dependent on biochemical, physiological, genetical and ecological factors. Insects have obligatory relationship with microbes for their survival and fitness to various environments. The role of endosymbiotic bacteria and fungi on manipulation of host reproduction, nutrition and provide defense against pathogens was reported by Feldhaar and Gross (2009), Gibson and Hunter (2010) and that confer resistance to insecticides by Indiragandhi *et al.* (2007) Hemalatha *et al*. (2012), Kikuchi *et al*. (2012), Snehaa *et al*. (2013). The present study revealed that there was more number of gut microflora associated with the insects of Dharwad where the insecticide usage pattern and the number of sprays were very high as compared to other locations (Table 1). The persistence of insects followed by insecticide failure at Dharwad may be due to development of resistance by high number of gut

microflora. More over more number of *Bacillus* spp. were recorded in Dharwad population. Broderick *et al*. (2004) reported that *Bacillus* spp. were reported to play an important role in growth and development of insects. A good diversity of microflora was recorded across the location and is unique with respect to the location. None of them are repeated except the genera *Bacillus* and *Enterobacter.* The obligate association of microorganism with the insect and the complete dependence of insect on its microorganisms pave a way for developing novel insect pest management strategies. The association between endosymbionts and leafhoppers especially *A. biguttula biguttula* is very poorly understood and that the unexploited association and their biology could make significant contribution to control the insect pests. The present study characterised an array of bacterial genera from the gut of *A. biguttula biguttula* for the first time and are in accordance with the earlier reports from other insect families by Moore (1972), Broderick *et al*. (2004), Xiang *et al*. (2006) and Indiragandhi *et al*. (2011).

In the current study an array of bacteria belonging to different genus and species were characterized based on 16S rRNA sequences. Rajagopal (2009) reported that the bacterial distribution and diversity in many insects were studied based on 16S rRNA sequences. More number of bacteria were characterized from the gut of insects which have been exposed to more rounds of insecticides sprays, viz. Guntur, Dharmapuri and Dharwad as compared to Raichur, Almora, Someshwar where less number of sprays were done (Table 2). Further it was observed that more number of bacteria were characterized from the insects which have been exposed to combination of insecticides spray as compared to single insecticide spray. Dowd (1991) documented that the symbionts appeared to be involved in the detoxification of certain insecticides and the enzymes involved in detoxification were esterases, glycosidases, lipases, proteases, phosphatases and glutathione transferases, 1 naphthyl acetate esterases. In the present study we have characterized the culturable bacteria, viz. *Pseudomonas geniculata***,** *Stenotrophomonas maltophilia, Serratia marcescens, Enterobacter* sp, *Paenibacillus* sp. which probably involve in the fitness attributes of the insects. The enzymes and other secondary metabolites produced by gut bacteria *Pseudomonas* sp., *Stenotrophomonas* sp., *Acinetobacter* sp. and *Serratia marcescens* associated with diamondback moth may involve in the host nutrition, defense against pathogens and insecticide resistance reported by Indiragandhi *et al.* (2007). Liquid chromatography mass spectrometry (LCMS) studies of Hemalatha *et al*. (2012) revealed that midgut bacteria *Enterobacter* spp. and *Paenibacillus* sp. isolated from *Chrysoperla zastrowi sillemi* larvae was able to degrade the acephate and indoxocarb. The present study also characterized the association of *Enterobacter asburiae, Enterococcus silesiacus* from the guts of leafhoppers of Guntur (Table 1) which was exposed to nine rounds of insecticides sprays as compared to Bangalore which was not exposed to insecticides at all. *Enterobacter hormaechei* which was isolated from the insects of Bangalore is different from the Guntur and probably *E. asburiae* and *E. silesiacus* of Guntur may involve in the detoxification of insecticides. In the present study, *Paenibacillus cineris* was isolated from the guts of leafhoppers of Almora which is in accordance with the report of Hemalatha *et al*. (2012). These organisms may be playing important role in conferring resistance to pesticides. Kukuchi *et al*. (2012) reported that large numbers of fenitrothion degrading symbionts (*Burkholderia* sp.) were isolated from the guts of bean bugs *Riptortus pedestris.* The occurrence of common in-habitants, viz. *Bacillus* spp. *Serratia,* and *Pseudomonas* in many insect guts was reported by Broderick *et al*. (2004) and *Bacillu*s spp. was reported to play an important role in growth and development of insects. Archana *et al.* (2006) reported that *Pantoea* spp., which was commonly found in *Dendroctonus frontalis* larvae, might be involved in nitrogen fixation and detoxification to confer release of defensive compounds known to be metabolized by bacteria. The occurrence of common gut bacterial genera *Enterococcus, Serratia, Enterobacter*, *Staphylococcus, Paenibacillus, Pantoea and Bacillus* in the insects of various crops and their role in the host fitness attributes was reported by Broderick *et al*. (2004). The present study also revealed the occurrence of these genera in the gut of leafhopper and the exact role played by them will be investigated in future studies.

Farmers were unable to control the cotton leafhoppers in certain cotton growing regions in spite of targeting this insect with several rounds of various combination of insecticides. It seems that the insects might have developed resistance against the insecticides. Insects were collected from such locations and explored for the isolation of gut microflora. Thirty culturable bacteria were isolated and characterized through 16S rDNA analysis from the guts of sixteen live populations of *A. biguttula biguttula* which have been exposed several rounds of insecticides. The predominant culturable bacteria associated with the insects were, viz. *Pseudomonas geniculata***,** *Stenotrophomonas maltophilia, Serratia marcescens, Enterobacter* sp, *Paenibacillus* sp., *Bacillus* sp. which probably involve in the fitness attributes of the insects including insecticide resistance. Diverse gut microflora with more *Bacillus* spp. associated with the insects of Dharwad where the insecticide usage pattern and the number of sprays were very high as compared to other locations. The bacterial genera *Enterobact*or and *Paenibacillus* have been characterized from the insecticide exposed insects might probably developed insecticide resistance. Association and the role of bacterial communities in the insect gut were frequently reported and received much attention, while other microbial groups are sparse. The culturable bacteria establish a specific and beneficial symbiosis with the insects and confer resistance to the host insects against insecticides. The present study revealed the occurrence of several bacteria in the gut of *A. biguttula biguttula.* Further studies

are required to pinpoint the role played by these bacteria for the fitness attributes including insecticide resistance which will be useful for taking suitable pest management practices against cotton leafhopper *A. biguttula biguttula*.

ACKNOWLEDGEMENTS

Authors are grateful to Dr C A Virakthmath, Professor Emeritus, Entomology, UAS, GKVK, Bangaluru, India and Dr J Poorani, NBAII, Benglaluru, India for identifying the insect. We thank Mrs Madhusmita, Senior Research Fellow, NBAII for helping to analyse the phylogenetic relationship.

REFERENCES

Anonymous. 2015. Report of Cotton Corporation of India Ltd, Mumbai, web page.

Archana V, Italo Delalibera J R, Jo H, Kier D K, Patrick D S and Kenneth F R. 2006. Characterization of gut-associated bacteria in larvae and adults of the southern pine beetle, *Dendroctonus frontalis Zimmermann. Environmental Entomology* **35:** 1 710—7.

Banerjee S K, Turkar K S and Wanjari R R. 2000. Evaluation of newer insecticides for the control of bollworms in cotton. *Pestology* **24**: 14—6.

Boush G M and Matsumura F.1967. Insecticidal degradation by *Pseudomonas melophthora*, the bacterial symbionts of the apple maggot. *Journal Economic Entomology* **60**: 918—20.

Broderick N A, Raffa K F, Goodman R M and Handelsman J.2004. Census of the bacterial community of the gypsy moth larval midgut by using culturing and culture independent methods. *Applied Environmental Microbiology* **70**: 293—300.

Dowd P F. 1991. Insect fungal symbionts: a promising source of detoxifying enzymes, *Journal of Industrial Microbiology* **9**: 149—61.

Feldhaar H and Gross R. 2009. Insects as hosts for mutualistic bacteria. *International Journal of Medical Microbiology* **299**: 1—8.

Felsenstein J.1985. Confidence limits on phylogenies: An approach using the bootstrap. *Evolution* **39**: 783—91.

Feng W, Wang X Q, Zhou W, Liu G Y and Wan Y J. 2011. Isolation and characterization of lipase producing bacteria in the intestine of the silkworm, *Bombyx mori*, reared on different forage. *Journal of Insect Science* **11**:135.

Genta, F A, Dillon R J, Terra W R and Ferreira C. 2006. Potential role for gut microbiota in cell wall digestion and glucoside detoxification in *Tenebrio molitor* larvae. *Journal of Insect Physiology* **52**: 593—601.

Gibson C M and Hunter M S. 2010. Extraordinary widespread and fantastically complex: Comparative biology of endosymbiotic bacterial and fungal mutualists of insects. *Ecological Letter* **13**: 223—34.

Harley J P and Prescott L M. 2002. *Laboratory Exercises in Microbiology,* Fifth edn. The McGraw Hill Companies.

Hemalatha B N, Reetha B, Venkatesan T, Jalali S K and Sriram S. 2012. Degradation of insecticide by *Enterobacter* sp. and *Paenibacillus* sp. isolated from midgut of *Chrysoperla zastrowi sillemi* Larvae. (*In*) *International Conference on Plant Health Management for Food Security,* Hyderabad, India, p. 104

Indiragandhi P, Anandham R, Madhaiyan M, Poonguzhali S, Kim G H, Saravanan V S and Sa T M. 2007. Cultivable bacteria associated with larval gut prothiofos-resistant, prothiofos-susceptible and field-caught populations of diamondback moth, *Plutella xylostella* and their potential for, antagonism towards entomopathogenic fungi and host insect nutrition. *Journal of Applied Microbiology* 103: 2664—75.

Indiragandhi P, Anandham R and Sa T M. 2011. Functional significance of insect gut bacteria and their role in the host insect processes, development and crop production. *Bacteria in Agrobiology: Plant growth responses,* pp 309-35 Maheshwari DK (Ed.) Springer-Verlag, Berlin, Heidelberg.

Kikuchi Y, Hayatsu M, Nagayama A, Tago K and Fukatsu T. 2012. Symbiont mediated insecticide resistance. *Proceedings of National Academy of Sciences,* USA **109**: 8 618—22.

Kimura M. 1980. A simple method for estimating evolutionary rate of base substitutions through comparative studies of nucleotide sequences. *Journal of Molecular Evolution* **16**:111—20.

Kranthi K R. 2007. Insecticide resistance management in cotton to enhance productivity. Model training course on " cultivation of long staple cotton (ELS), 15-22, December, Central Institute for Cotton Research, Regional station, Coimbatore, pp 214—31.

Kshirsagar S D, Satpute N S and Moharil M P. 2012. Monitoring of insecticide resistance in Cotton leafhoppers, *Amrasca bigutulla bigutulla* (Ishida). *Annals of Plant Protection Sciences* **20**: 283—6.

Madhusudan S, Jalali S K, Venkatesan T, Lalitha Y and Srinivas R P. 2011. 16S rRNA gene based identification of gut bacteria from laboratory and wild larvae of *Helicoverpa armigera* (Lepidoptera: Noctuidae) from tomato farm. *Bioscan* **6**: 175—83.

Mohan M and Gujar G T. 2003. Local variation in susceptibility of diamondback moth, *Plutella xylostella* (Linnaeus) to insecticides and role of detoxification enzymes. *Crop Protection* **22**:495—504.

Moore G E. 1972. Microflora from the alimentary tract of healthy southern pine beetles, *Dendroctonus frontalis* (Scolytidae), and their possible relationship to pathogenicity, *Journal of Invertebrate Pathology* **19**: 72—5.

Nakabachi A and Ishikawa .1999. Provision of riboflavin to the host aphid, *Acyrthosiphon pisum*, by endosymbiontic bacteria *Buchnera, Journal of Insect Physiology* **45**:1—6.

Praveen P M. 2003. 'Studies on insecticide resistance in early season sucking pests of Cotton in Tamil Nadu.' Ph D thesis, Tamil Nadu Agricultural University, Coimbatore, India, p 116.

Peshin R, Bandral R S, Dhawan A K, Zhang W, Wilson L and Dhawan A K. 2009. Integrated pest management: History, overview and principles of ecologically based pest management. *Integrated Pest Management: Innovation-development Process* pp—26. Peshin P and Dhawan A K (Ed.). Springer Sciences and Business.

Rajagopal R. 2009. Beneficial interactions between insects and gut bacteria. *Indian Journal of Microbiology* **49**: 114—9.

Ram Singh and Jaglan R S. 2005. Development and management of insecticide resistance in cotton whitefly and leafhopper-review. *Agricultural Review* **26** (3): 229—34.

Robyn J R, Colin S, Colin J J, Rinku P, Gunjan P, Matthew C T, Christopher W C, Jian- Wei L and John G O. 2011. The evolution of new enzyme function: lessons from xenobiotic metabolizing bacteria versus insecticidal insects. *CSIRO* **4**: 225-48.

Sarfraz M, Dosdall L M and Keddie B A. 2006. Diamondback moth-host plant interactions: implications for pest management. *Crop Protection* **25**: 625—39.

Snehaa C P, Hemalatha B N, Reetha B, Venkatesan T and Jalali S K. 2013. Molecular characterization of microbes associated with cotton mealy bug, *Phenacoccus solenopsi* Tinsley. (In) *New Horizons in Insect Science, International Conference on Insect Science (ICIC 2013),* 14-17 February, Bangalore, India.

Srinivasa Murthy K, Rajeshwari R, Venkatesan T and Nesil L B. 2011. Detection and characterization of *Wolbachia* in *Cotesia plutellae* (Kurdjumov) (Hymenoptera: Braconidae), a parasitoid of the diamond back moth *Plutella xylostel* (Linn.). *Journal of Biological Control* **25:** 213—6.

Tamura K, Peterson D, Peterson N, Stecher G, Nei M and Kumar S. 2011. MEGA5: Molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. *Molecular Biology and Evolution:* **28**(10): 2 731—9

Thompson J D, Higgins D G and Gibson T J. 1994. CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. *Germa Nucleic Acids Research* 4 673—80.

Vuilleumier S. 1997. Bacteria glutathione S-transferase: what are they good for? *Journal of Bacteriology* **179**: 1 431—41.

Werren J H. 2012. Symbionts provide pesticide detoxification. *Proceedings of National Academy of Sciences, USA,* **109** (22): 8 364—5.

Xiang H, Wei G H, Jia S, Huang J, Miao X X, Zhou Z, Zhao L P and Huang, Y P. 2006. Microbial communities in the larval midgut of laboratory and field populations of cotton bollworm (*Helicoverpa armigera*). *Canadian Journal of Microbiology* **52**: 1 085—92.